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RESEARCH ARTICLE

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Antibiotic resistance patterns of bacteria isolated from *Clarias gariepinus* farms in Kaduna state, Nigeria

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ABSTRACT

Fish farming is increasing globally, with an increase in bacterial infections known to cause morbidity and varying mortality, affecting the productivity and profitability of aquaculture. The objective of this study was to determine the antibiotics susceptibility and multiple antibiotic resistance index of bacteria isolated from fish in some selected fish farms in Kaduna State to ten commonly used antibiotics using the Kirby-Bauer disc diffusion method. In total, 84 bacteria were isolated from 75 *Clarias gariepinus* in this study, belonging to 12 genera. The antibiotic profile of the bacteria isolated displayed different sensitivity and resistance to the antibiotics used. The highest numbers of the Gram-positive (59.5%) and Gram-negative (69%) bacteria, respectively, were sensitive to ciprofloxacin compared to the other antibiotics. All the bacterial isolates displayed varying diversity of multidrug-resistant patterns. A total of 38 and 41 different resistance patterns for Gram-positive and Gram-negative respectively were observed. The multiple antibiotic resistance (MAR) index analysis reveals that 97.3% of the bacteria had a high MAR index value (> 0.2). In conclusion, there is a diversity of bacteria organisms within the fish farms that are pathogenic to both fish and humans. Therefore, there is a need to implement optimal preventive management measures and control the use of antibiotics.

Keywords

Antimicrobials, aquaculture, health risk, multidrug resistance, pathogens

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Abbreviations

C. gariepinus: *Clarias gariepinus*
E. coli: *Escherichia coli*
MAR: Multiple antibiotic resistance
AMP: Ampicillin
CIP: Ciprofloxacin
FFC: Florfenicol

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Introduction

Fish production through aquaculture provides an alternative supply of fish for human consumption [1, 2]. This has led to an increase in fish production levels to meet the protein demand of the growing population [3, 4]. In bridging the demand and supply gap of fish, *Clarias gariepinus* is a suitable choice for aquaculture in Africa, especially Nigeria, owing to its hardy nature and wide acceptability [5]. However, increasing demand for fish is associated with the intensification of fish farming activities, such as increased stocking density, and a rise in water quality challenges, which facilitate a higher incidence of disease outbreaks [6, 7]. Furthermore, the occurrence of various types of diseases, most of which are caused by bacteria, at any stage of fish culture has a significant impact on the economic viability of fish farms [8, 9]. Consequently, this has led to the use of antibiotics as a growth promoter, for prophylactic and therapeutic purposes [10, 11]. Excessive use of antibiotics in aquaculture in many countries has been attributed to the development and dissemination of antibiotic-resistant bacteria [12, 13, 14].

Assessing and monitoring antimicrobial-resistant bacteria from fish for human consumption from different parts of the world is needed regularly to evaluate and detect the emergence, trend, and changes in the resistance pattern towards antimicrobial drugs [15, 16]. Therefore, this study is aimed at isolating and identifying bacteria from *Clarias gariepinus* in some selected fish farms in Kaduna State, Nigeria, and determining their antimicrobial susceptibility and resistance pattern to 10 commonly used antibiotics.

Results

A total of 84 bacteria belonging to 12 genera were isolated from 75 *Clarias gariepinus* samples from this study. Out of 42 bacteria isolated, 16 (19.0%) were *Bacillus subtilis*, 3 were (3.6%) *Corynebacteria aquaticum*, 17 were (20.3%) *Staphylococcus aureus,* and 6 were (7.1%) *Streptococcus agalactiae*. Forty-two Gram-negative bacteria were also isolated, which consisted of *Aeromonas hydrophila* (2.4%, n = 2), *Citrobacter freundi* (4.8 %, n = 4), *Escherichia coli* (13.1%, n= 11), *Klebsiella pneumoniae* (3.6%, n = 3), *Proteus mirabilis* (11.9%, n = 10), *Pseudomonas aeruginosa* (7.1%, n = 6), and *Salmonella enterica* and *Shigella* species (3.6%, n = 3) (Figure 1). *Staphylococcus aureus* (20.3%) was the most prevalent species followed by *Bacillus subtilis* (19.0%) and *E. coli* (13.1%). *Aeromonas hydrophila* was the least prevalent (2.4%) bacteria isolated (Figure 1).

The antibiotic profile of the bacteria isolated revealed different sensitivity and resistance to the ten antibiotics used. The majority of the Gram-positive bacteria (59.5%) were sensitive to ciprofloxacin. Among the Gram-positive bacteria, no level of sensitivity was detected to Vancomycin. With a *Chi*-Square value of 80.30, the difference in the sensitivity level of antibiotic susceptibility was significant at $p \leq 0.01$. There was no significant difference ($p = 0.27$) in susceptibility to different antibiotics. There was a level of resistance to all the antibiotics used, with vancomycin causing the highest level of resistance to the Gram-positive bacteria. There was a significant statistical difference ($p \leq 0.01$) in the resistance of the Gram-positive bacteria to different antibiotics (Table 1).

The Gram-negative bacteria were mostly susceptible to Ciprofloxacin (69.0%) and showed the lowest (4.8%) level of susceptibility to penicillin. The level of sensitivity to other antibiotics ranged between gentamicin (66.7%) and ampicillin (7.1%). There was a significant difference ($p \leq 0.01$) in the sensitivity level to antibiotics in the Gram-negative bacteria. In addition, there was a significant difference ($p \leq 0.01$) between the antibiotics in terms of their intermediate and resistance profiles with the Gram-negative bacteria. (Table 2). All the bacterial isolates displayed varying diversity of multidrug-resistant patterns to more than one antibiotic. There were differences in the multidrug-resistance profiles of the bacteria within the different species of the isolates. The prevalence of multidrug resistance was 97.6% for both Gram-positive and Gram-negative bacteria respectively and was resistant to more than two antibiotics. From the Gram-positive, one of the isolates was resistant to two antibiotics 1(2.4%), 4 (9.52 %) were resistant to three antibiotics, 6 (14.29%) were resistant to four antibiotics, 13 (30.92%) were resistant to five antibiotics, 11 (26.1%) were resistant to six antibiotics, 4 (9.52 %) were resistant to seven antibiotics, and 3 (3.74%) were resistant to eight antibiotics out of the ten antibiotics used. A total of 38 different resistance patterns were observed. The multidrug resistance patterns for Gram-positive bacteria isolated from *Clarias gariepinus* showed a significant difference ($p \leq 0.01$) with a Chi-Square value of 22.56. The highest prevalence was recorded among the 5 antibiotics combinations (30.9%), with the double antibiotic combinations having the least

Abbreviations-Cont'd

CN: Gentamicin
OXE: Oxytetracycline
OX: Oxacillin
P: Penicillin
S: Streptomycin
TE: Tetracycline
VA: Vancomycin
SEM: Standard error of the mean

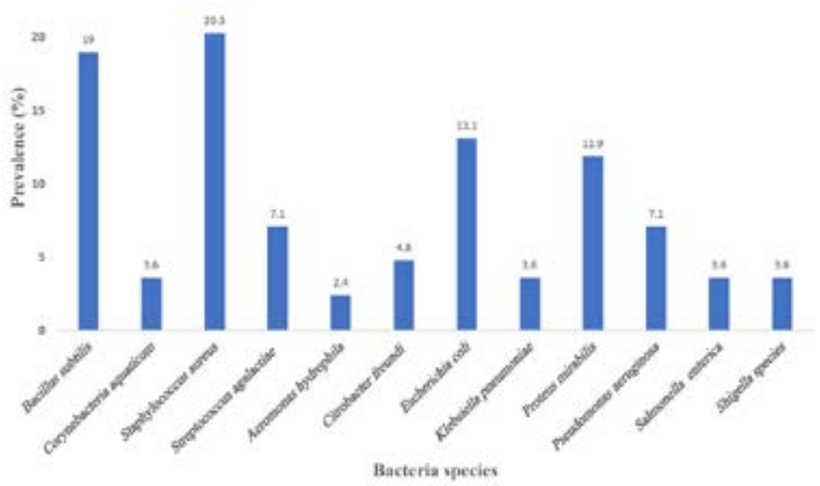

Figure 1.
Prevalence of Gram-positive and Gram-negative bacteria isolates from Gills of *Clarias gariepinus* from fish farms

(2.4%) (Table 3).

For the Gram-negative bacteria, one of the isolates was resistant to two and three antibiotics, respectively. Eight (19.04%) were resistant to four antibiotics, 18 (42.86%) were resistant to five antibiotics, 9 (21.43%) were resistant to six antibiotics, 4 (9.52%) were resistant to seven antibiotics and 1(2.4%) was resistant to eight antibiotics out of the ten antibiotics used. A total of 41 different resistance patterns were observed for Gram-negative in this study. The multidrug resistance patterns and MAR Index of Gram-negative bacteria from *Clarias gariepinus* are presented in Table 4. The highest prevalence of multidrug resistance patterns was seen in the five antibiotic combinations (42.9%) with a MAR value of 0.5. The difference between the multidrug resistance patterns was significant ($p \leq 0.01$) with a Chi-Square value of 45.89 (Table 4).

Table 1.
Percentage distribution of antibiotics susceptibility of Gram-positive bacteria isolates from Gills of *Clarias gariepinus* from some fish farms in Kaduna State, Nigeria

Antibiotic	N (µg)	Sensitive (%)	χ^2	*p*	Intermediate (%)	χ^2	*p*	Resistance (%)	χ^2	*p*
Ciprofloxacin	5	25 (59.5)			6 (14.3)			11 (26.2)		
Gentamicin	10	21 (50.0)			8 (19.0)			13 (31.0)		
Florfenicol	30	20 (47.6)			6 (14.3)			16 (38.1)		
Streptomycin	10	10 (23.8)			14 (33.3)			18 (42.9)	49.36	
Tetracycline	30	9 (21.4)	80.30	<0.01#	14 (33.3)	11.08	0.27	19 (45.2)		<0.01#
Oxytetracycline	30	7 (16.7)			9 (21.4)			26 (61.9)		
Oxacillin	1	7 (16.7)			6 (14.3)			29 (69.0)		
Ampicillin	10	6 (14.3)			9 (21.4)			27 (64.3)		
Penicillin (in units)	10	2 (4.8)			10 (23.8)			30 (71.4)		
Vancomycin	30	0 (0.0)			10 (23.8)			32 (76.2)		
Total		107 (25.5)			92 (21.9)			221 (52.6)		

N = Concentration of antibiotics used; χ^2 = *Chi* Square test; # = Significant at $p < 0.05$

Table 2.
Percentage distribution of antibiotics susceptibility patterns of Gram-negative bacteria isolates from Gills of *Clarias gariepinus* from some fish farms in Kaduna State, Nigeria

Antibiotic	N (µg)	Sensitive (%)	χ^2	*p*	Intermediate (%)	χ^2	*p*	Resistance (%)	χ^2	*p*
Ciprofloxacin	5	29 (69.0)			5 (11.9)			8 (19.0)		
Gentamicin	10	28 (66.7)			3 (7.1)			11 (26.2)		
Florfenicol	30	26 (61.9)			4 (9.5)			12 (28.6)		
Streptomycin	10	11 (26.2)			13 (31.0)			18 (42.9)		
Tetracycline	30	8 (19.0)	121.10	< 0.01#	13 (31.0)	22.61	0.01#	21 (50.0)	75.12	< 0.01#
Oxytetracycline	30	6 (14.3)			12 (28.6)			24 (57.1)		
Ampicillin	10	3 (7.1)			11 (26.2)			28 (66.7)		
Vancomycin	30	5 (11.9)			7 (16.7)			30 (71.4)		
Oxacillin	1	6 (14.3)			5 (11.9)			31 (73.8)		
Penicillin (in units)	10	2 (4.8)			5 (11.9)			35 (83.3)		
Total		124 (29.5)			78 (18.6)			218 (51.9)		

N = Concentration of antibiotics used; χ^2 = *Chi* Square test; # = Significant at $P < 0.05$.

Discussion

Bacteria are an important component of the aquatic environment, and the interplay between these organisms and the changes in the habitat of the fish will lead to the exacerbation of disease in the fish farms, thereby causing great economic losses [17]. The identification of bacteria from *C. gariepinus* is very important as it provides information on the level of contamination in the fish, the culture environment, and the risk of transfer of the pathogens to humans to cause diseases like cholera, dysentery, and salmonellosis [18]. In this study, twelve different genera of bacteria known to cause disease in both fish and humans were isolated from *C. gariepinus* in the study. However, it differed from the findings of Uddin and Al-Harbi [19], who isolated 10 bacteria genera from polycultured common carp (*Cyprinus carpio*) and African catfish (*Clarias gariepinus*), Danba et al [20] isolated 5 genera from *C. gariepinus* from selected fish farms in Kano, Wamala et al. [21], isolated 15 in *Oreochromis niloticus* (Nile tilapia) and *Clarias gariepinus* (African catfish) in Uganda with most of the bacteria genera reported by Uddin and Al-Harbi [19]. Danba *et al* [20] and Wamala *et al* [21] reported findings similar to the present study. The differences in the genera and species of bacteria observed may be due to the different geographical locations, culture environments, species of fish, and different sampling and isolation methods.

Gram-negative bacteria were the most prevalent bacterial isolates from this study. This is similar to the findings of Tsfaye *et al.* [22] and Kousar *et al.* [23]. The isolation of E. *coli, Klebsiella pneumoniae*, *Proteus* mirabilis, *Staphylococcus aureus, Streptococcus* agalactiae, and *Vibrio* species from *C. gariepinus* is an indication of fecal contamination from livestock manure used for pond fertilization and the indiscriminate deposition of human and animal excreta into ponds and rivers that harbor fish or through the washing of land surfaces into water bodies during the rainy season [24]. Free-roaming animals, especially dogs, birds, and ruminants in the mixed farming system, contribute to the fecal contamination of surface water and ponds [25, 26]. *Staphylococcus aureus* isolates which were hemolytic on blood agar are known to be pathogenic to fish and their presence could be due to contamination of the fish by fish handlers during feeding, handling activities, and harvesting as observed also by Afolabi *et al.* [18]. The high presence of *Proteus mirabilis* in fish farms has been reported by Wanja, *et al.* [27] and was attributed to the use of poultry litter for fertilization of the ponds. The presence of these microorganisms poses a serious public health threat as some of the bacterial organisms isolated in this study, such as *Aeromonas hydrophila, Citrobacter freundi,* and *Pseudomonas aeruginosa* are known to be pathogenic to humans and are etiological agents of infectious diseases in fish, leading to mortalities in association with unfavorable environmental conditions in intensive fish farms [27, 28].

In this study, the antibiogram showed that most of the bacteria species isolated showed varying resistance

Table 3.
Multidrug resistance patterns and MAR Index of Gram-positive bacteria isolated from *Clarias gariepinus* from some fish farms in Kaduna State, Nigeria

Resistance patterns	No. of antibiotics involved	MAR	No of isolates	Bacteria species involved	Prevalence (%)	χ^2	*p*
OX, P	2	0.2	1	*Streptococcus agalactiae*	1 (2.4)		
OX, P, FFC	3	0.3	1	*Bacillus subtilis*			
TE, VA, P	3	0.3	1	*Staphylococcus aureus*			
OX, VA, P	3	0.3	1	*Bacillus subtilis*	4 (9.5)		
VA, CN, AMP	3	0.3	1	*Staphylococcus aureus*			
VA, P, FFC, OXE	4	0.4	1	*Corynebacteria aquaticum*			
VA, P, S, FFC	4	0.4	1	*Staphylococcus aureus*			
OX, P, AMP, OXE	4	0.4	1	*Corynebacteria aquaticum*			
OX, VA, AMP, OXE	4	0.4	2	*Staphylococcus* and *Bacillus subtilis*	6 (14.3)		
TE, VA, FFC, CIP	4	0.4	1	*Bacillus subtilis*			
OX, VA, P, CN, FFC	5	0.5	1	*Streptococcus agalactiae*			
OX, TE, VA, P, AMP,	5	0.5	1	*Staphylococcus aureus*			
OX, P, S, AMP, CIP	5	0.5	1	*Staphylococcus aureus*			
OX, CN, S, AMP, OXE	5	0.5	1	*Staphylococcus aureus*			
OX, VA, P, FFC, OXE	5	0.5	1	*Staphylococcus aureus*			
OX, VA, P, S, AMP	5	0.5	2	*Staphylococcus aureus* and *Bacillus subtilis*	13 (30.9)		
OX, VA, P, S, OXE	5	0.5	1	*Bacillus subtilis*			
OX, VA, S, AMP, OXE	5	0.5	1	*Streptococcus agalactiae*		22.56	<0.01#
OX, TE, S, AMP, CIP	5	0.5	1	*Bacillus subtilis*			
TE, VA, P, AMP, OXE	5	0.5	1	*Bacillus subtilis*			
VA, P, AMP, FFC, OXE	5	0.5	1	*Bacillus subtilis*			
OX, TE, CN, AMP, FFC, OXE	6	0.5	1	*Bacillus subtilis*			
OX, TE, P, S, AMP, OXE	6	0.6	1	*Bacillus subtilis*			
OX, VA, P, AMP, FFC, OXE	6	0.6	2	*Bacillus subtilis* and *streptococcus agalactiae*			
OX, TE, CN, AMP, FFC, OXE	6	0.5	1	*Bacillus subtilis*			
OX, TE, P, S, AMP, OXE	6	0.6	1	*Bacillus subtilis*			
OX, VA, P, AMP, FFC, OXE	6	0.6	2	*Bacillus subtilis* and *streptococcus agalactiae*	11 (26.1)		
OX, VA, P, CN, CIP, OXE	6	0.6	1	*Bacillus subtilis*			
TE, VA, P, CN, AMP, OXE	6	0.6	1	*Staphylococcus aureus*			
TE, VA, P, CN, S, FFC	6	0.6	1	*Staphylococcus aureus*			
TE, VA, P, S, AMP, OXE	6	0.6	1	*Staphylococcus aureus*			
OX, TE, VA, CN, S, FFC, OXE	7	0.7	1	*Staphylococcus aureus*			
OX, TE, VA, P, S, AMP, FFC	7	0.7	1	*Bacillus subtilis*	4 (9.5)		
OX, TE, VA, P, S, CIP, OXE	7	0.7	2	*Staphylococcus aureus* and *Corynebacteria aquaticum*			
OX, TE, VA, P, CN, AMP, CIP, OXE	8	0.8	1	*Streptococcus agalactiae*			
OX, TE, P, CN, AMP, FFC, CIP,OXE	8	0.8	1	*Staphylococcus aureus*	3 (7.1)		
OX,VA,P,CN,AMP,FFC,CIP,OXE	8	0.8	1	*Staphylococcus aureus*			

AMP: Ampicillin; CIP: Ciprofloxacin; FFC: Florfenicol; CN: Gentamicin; OXE: Oxytetracycline; OX: Oxacillin; P: Penicillin; S:Streptomycin ;TE : Tetracycline ; VA: Vancomycin. Multiple antibiotics resistance (MAR); χ^2 =*Chi* Square test ; # = Significant at $p < 0.05$.

Table 4.
Multidrug resistance patterns and MAR Index of Gram-negative bacteria isolated from *Clarias gariepinus* in some fish farms in Kaduna State, Nigeria

Resistance patterns	No. of antibiotics involved	MAR	No of isolates	Bacteria species involved	Prevalence (%)	χ^2	*p*
OX, AMP	2	0.2	1	*Pseudomonas aeruginosa*	1 (2.4)		
VA, AMP, CIP	3	0.3	1	*Pseudomonas aeruginosa*	1 (2.4)		
OX, P, S, OXE	4	0.4	1	*E. coli*			
OX, TE, P, FFC	4	0.4	1	*E. coli*			
OX, TE, VA, P	4	0.4	1	*Proteus mirabilis*	8 (19.0)		
OX, TE, P, S	4	0.4	1	*Proteus mirabilis*			
OX, VA, P, AMP	4	0.4	1	*Salmonella enterica*			
OX, VA, CIP, OXE	4	0.4	1	*Klebsiella pneumoniae*			
TE, VA, S, OXE	4	0.4	1	*E. coli*			
VA, P, AMP, OXE	4	0.4	1	*E. coli*			
OX, TE, VA, P, S	5	0.5	1	*Citrobacter freundi*			
OX, VA, P, S, FFC	5	0.5	1	*Proteus mirabilis*			
OX, TE, VA, P, AMP,	5	0.5	1	*Aeromonas hydrophila*			
OX, P, CN, FFC, OXE	5	0.5	1	*Salmonella enterica*			
OX, TE, P, CN, AMP,	5	0.5	1	*Proteus mirabilis*			
OX, VA, CN, AMP, OXE	5	0.5	1	*Citrobacter freundi*			
OX, VA, P, FFC, OXE	5	0.5	1	*Proteus mirabilis*			
OX, VA, P, AMP, OXE	5	0.5	1	*Shigella* species	18 (42.9)		
OX, VA, P, S, AMP	5	0.5	1	*E. coli*			
OX, VA, P, CIP, OXE	5	0.5	1	*Shigella* species	45.89	<0.01#	
OX, VA, P, CN, AMP	5	0.5	1	*Citrobacter freundi*			
OX, P, AMP, CIP, OXE	5	0.5	1	*Pseudomonas aeruginosa*			
TE, VA, P, AMP, OXE	5	0.5	1	*Aeromonas hydrophila*			
TE, VA, P, S, OXE	5	0.5	1	*Pseudomonas aeruginosa*			
TE, VA, S, AMP, FFC	5	0.5	1	*Klebsiella pneumoniae*			
VA, P, CN, AMP, OXE	5	0.5	1	*Klebsiella pneumoniae*			
TE, P, CN, CIP, OXE	5	0.5	1	*Proteus mirabilis*			
TE, P, S, AMP, OXE	5	0.5	1	*E. coli*			
OX, VA, P, CN, AMP, FFC	6	0.6	1	*Pseudomonas aeruginosa*			
OX, TE, P, S, AMP, FFC	6	0.6	1	*E. coli*			
OX, TE, VA, P, AMP, OXE	6	0.6	1	*Pseudomonas aeruginosa*			
OX, VA, CN, S, AMP, OXE	6	0.6	1	*Proteus mirabilis*			
OX, VA, P, CN, S, OXE	6	0.6	1	*E. coli*	9 (21.4)		
OX, VA, P, S, AMP, OXE	6	0.6	1	*Proteus mirabilis*			
TE, VA, P, CN, S, OXE	6	0.6	1	*E. coli*			
TE, VA, P, S, AMP, FFC	6	0.6	2	*Proteus mirabilis, Shigella* species			
OX, TE, P, AMP, FFC, CIP, OXE	7	0.7	1	*E. coli*			
OX, TE, P, CN, FFC, CIP, OXE	7	0.7	1	*E. coli*			
OX, TE, P, S, AMP, CIP, OXE	7	0.7	1	*Salmonella enterica*	4 (9.5)		
OX, TE, VA, P, S, AMP, OXE	7	0.7	1	*Citrobacter freundi*			
OX, VA, P, S, AMP, FFC, CIP,OXE	8	0.8	1	*Proteus mirabilis*	1 (2.4)		

AMP: Ampicillin; CIP: Ciprofloxacin; FFC: Florfenicol; CN: Gentamicin; OXE: Oxytetracycline; OX: Oxacillin; P: Penicillin; S: Streptomycin ;TE : Tetracycline ; VA: Vancomycin. Multiple antibiotics resistance (MAR) ;χ^2 = *Chi* Square test; # = Significant at $p < 0.05$.

to penicillin, oxacillin, vancomycin, ampicillin, oxytetracycline, and tetracycline, but they were found to be sensitive to ciprofloxacin, gentamycin, and florfenicol, which was similar to the findings of [29, 30]. The susceptibility of the bacteria to ciprofloxacin, gentamycin, and florfenicol might be due to the less frequent utilization of these antibiotics in aquaculture. The resistance of the bacteria species could be due to the extensive and indiscriminate use of drugs like vancomycin, ampicillin, oxytetracycline, and tetracyclines which are easily accessible over-the-counter antibiotics and have been the hallmark of antimicrobial treatment administered either in feeds or in baths in fish farming [31]. More so, several of these drugs are non-biodegradable, leading to an increase in selective pressure and thus has resulted in an increase in the occurrence of drug resistance in fish-pathogenic bacteria [32]. The high prevalence of antibiotic resistance observed in Gram-positive bacteria in this study has also been reported by Ayadiran and Dahunsi [33], who reported the highest rate of multiple antibiotic resistance in Gram-positive bacteria. This could be due to the ubiquitous nature of the Gram-positive bacteria in the culture environment of the fish.

Multi-antibiotic resistance (MAR) indexing is well known as an efficient and less expensive method of tracking bacteria sources [34]. As a result, the MAR index is a useful method of ascertaining the risk of pollution that could threaten the life of an animal [35]. The multidrug resistance (MDR) of the isolates was identified by observing the resistance pattern of the isolates to the antibiotics used. Varying antibiotic resistance patterns were observed for the different species of bacteria isolated in the study area. However, it was observed that bacterial species of the same genus displayed different antibiotic resistance patterns. Antibiotic resistance patterns may vary depending on the geographical location, management practice, and selective pressure [35], and these patterns change rapidly from time to time. The different patterns exhibited by different strains or species suggest how complex the understanding of the antibiotic's resistance is in the study area.

The MAR index analysis reveals that 97.3% of both Gram-positive and Gram-negative bacteria had a high MAR index value (> 0.2). This was similar to the findings of Kathleen *et al.* [35] and Adinortey *et al.* [31]. The high MAR index recorded indicates high contamination with antibiotics. The difference in the MAR index shows the impact of the use of antibiotics in the sampled fish farms. Diseases caused by bacteria with a high MAR index will be a great challenge to curb, leading to high mortalities and reduced profit on investment. Because bacteria possess multiple resistance mechanisms, this will aid in the reduction of antibiotic activity for both prevention and therapeutic purposes [36]. More so, the observed trend of multidrug-resistant strains poses a major public health concern globally, and there is a need to come up with effective policies and implementation plans to address these concerns.

In conclusion, the results from this study revealed the diversity of bacteria organisms within fish farms that are pathogenic to both fish and humans, which may pose a serious public health challenge to consumers when the fish are not properly cooked or handled. There is a high prevalence of antibiotic resistance, which may have environmental, public health, and global implications. Therefore, there is a need to implement optimal and more strict preventive management measures in fish farms that will prioritize adherence to practices as this will go a long way to helping produce healthy and wholesome fish as well as boost productivity. Controlled use of antibiotics in fish farming is very important, to avoid the occurrence and spread of antibiotic resistance and further complicate clinical management of the disease. Consequently, it has been strongly recommended that programs to monitor and regulate the usage of antimicrobial agents and the occurrence of antimicrobial resistance be advocated.

Materials & Methods

Study area and design

The study was carried out in Kaduna State, which is located at a geographic coordinate of latitude 10° 36' 33.54"N and Longitude 7° 25' 46.2144"E located in the northwestern part of Nigeria. It approximately occupies a total landmass of 48,473.2 square kilometers and has a population of more than 6 million people [37]. A cross-sectional study involving multistage random sampling of 15 active, grow-out fish farms from four local government areas (Sabo Gari, Kaduna North, Kaduna South, and Zaria Local Government Areas) of Kaduna State were sampled. Sampling was carried out based on the convenience and willingness of the fish farmers to participate in the study.

Fish sample collection

Samples of seventy-five live *Clarias gariepinus* (*C. gariepinus*), five fish per farm, were randomly selected from active productive grow-out farms within the study area. *C. gariepinus* fish with different total lengths of ≥ 12-35 cm and weights of 350 g – 1 kg were included in the study. The fish were caught using a fishnet from earthen ponds, plastic and concrete tanks between the hours of 06: 00 and 08:00 and put into a plastic bucket with a perforated cover containing water to ensure the survival of the fish samples.

They were later transported to the microbiology laboratory of the Faculty of Veterinary Medicine, Ahmadu Bello University, Zaria, for further processing within 2 hours post-collection.

Each live fish was sacrificed (by brain spiking to minimize suffering) and placed on a clean stainless tray dorsally, and a swab (sterile cotton wool soaked in 70% alcohol) was used to clean the fish from the operculum to the abdominal area to reduce bacterial load. The operculum of the fish was lifted to expose the gills, and swabs of the gills were taken for bacterial isolation using sterile swab sticks.

Isolation and identification of bacterial isolates

The examination was conducted to isolate, identify, and confirm bacterial isolates from *Clarias gariepinus*. Conventional methods of bacterial isolation, such as growth and morphology on selective media, were employed. The sterile swab sticks were used to swab the gills of the sampled fish and were put into the nutrient broth and incubated at 37 °C for 24 hrs for the growth of microorganisms. After incubation, a loopful of the sample was picked with a sterilized loop and streaked on the Nutrient agar plate for the isolation and purification of bacteria colonies. MacConkey agar plate was used to grow Gram-negative organisms and to distinguish between lactose fermenter and non-lactose fermenter bacteria. Eosin methylene blue agar (Oxoid, UK) was used for the isolation of *E.* coli, *Citrobacter* species, and *Klebsiella* species. *Salmonella Shigella* agar (Oxoid, UK) for *Salmonella and Shigella* species [38, 39]. The agar plates were then incubated for 18-24 hours at 37 °C, and subculturing of the discrete colonies from the different agar plates onto fresh agar plates was carried out aseptically to obtain pure colonies of isolates. The hemolytic activity of the bacteria was determined on blood agar. The bacteria were then identified using morphological characteristics, Gram staining, and biochemical tests such as motility test, oxidase test, catalase test, triple sugar iron (TSI), indole test, urease test, citrate utilization test, methyl red test, oxidative fermentation test, Voges Proskauer test, nitrate reduction test, and gelatin liquefaction test [39]. All reagents for biochemical tests were prepared according to manufacturer instructions (Difco ®, Laboratories, USA and Oxoid ®, London, UK) and the results were interpreted using the manual for bacteria identification [38] and online ABIS (Advanced Bacteriological Identification Software) [40]. Antibiotic susceptibility test

The susceptibility to antimicrobial drugs was carried out on each of the identified bacterial isolates using the disc diffusion method on Mueller-Hinton agar plates (MHA) (Oxoid Basingstoke, UK) with inocula adjusted to an optical density of 0.5 McFarland standard unit [40]. Pure bacterial isolates were inoculated into the nutrient broth and incubated at 37 °C for 24 hrs. After that, the growth in the nutrient broth was inoculated and swabbed on Mueller-Hinton agar plates. Ten common antibiotics, including ampicillin (10 µg), ciprofloxacin (5 µg), florfenicol (30ug), gentamycin (10 µg), oxacillin (5 µg), oxytetracycline (30 µg), penicillin (10 units), streptomycin (10 µg), tetracycline (30 µg), and vancomycin (30 µg), were dispensed on the swabbed plate using an automatic multi-disc dispenser (Bioanalyse) and incubated at 37 °C, for 18–36 h [42]. All the antibiotic discs used were supplied by Oxoid, UK. The results of the antibiotic susceptibility test were interpreted following standard measurement of zones of inhibition from the back of the agar plate to the nearest mm using a ruler and were interpreted as sensitive (S), intermediate (I), or resistant (R) according to the Clinical Laboratory Standard Institute [41].

Multiple Antibiotic Resistance (MAR) Index

The MAR index for each bacterial isolate was determined from the results of the disc diffusion method. It was calculated by dividing the total number of antibiotics to which the bacteria isolates were resistant by the total number of antibiotics used on the isolates. Multi-drug resistance was defined as resistance greater than or equal to four antimicrobials [43].

Data analysis

Data from the isolates were used for the determination of the prevalence rates of the bacterial isolates. The percentage resistance of the bacteria was also calculated for each of the antibiotics. The prevalence rate of bacteria isolates was ascertained as the number of times the bacteria organism was identified over the total number of times all the bacteria species were identified. The resistance rates for each antibiotic were calculated. The degree of resistance for each antibiotic from the different farms was compared using the *chi*-squared and student's *t*-test. Values of $p < 0.05$ with a 95% confidence interval were considered significant. A one-way ANOVA with *Tukey's* posthoc test was performed to compare the differences in antibiotic-resistant bacteria from the various sources.

Authors' Contributions

AAD., LS., and SO conceived and designed the experiments. AAD. and SAA carried out the experiments. SMD analyzed the data. LS and SO provided research space and equipment. SMD and OMD Contributed reagents/materials/analysis tools. AAD took the lead in writing the manuscript. All authors provided critical feedback and helped shape the research, analysis and manuscript

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Competing Interests

The authors declare that there is no conflict of interest.

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